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A CASE OF NON-INHERITANCE OF FLUCTUATING VARIATIONS AMONG BACTERIA.*

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THE subject of variation among the bacteria is of obvious importance to those who are concerned with the study of these organisms in their relations to everyday life. The pathologist and the sanitarian and the soil bacteriologist are vitally concerned with the question as to how far the types with which they work are constant and invariable, and to what extent they may modify and interchange their characters. The problem has a much wider significance, however. The bacteria exhibit protoplasm in one of its simplest forms. The absence of amphotropism and the extent to which these organisms are exposed to the direct influence of the environment make it possible to study in them some of the fundamental problems of heredity and variation in their simplest terms. The rapidity with which bacteria multiply and the ease with which they are cultivated make it reasonable to prophesy that the study of bacterial variations will some day throw important light on such central problems of general biology as the origin of species.

There are two or three fundamental types of variations among the bacteria which ought to be distinguished as clearly as possible. In the first place many of the observed differences in bacterial cultures are not variations at all but temporary modifications of behavior due to the direct effect of existing conditions of cultivation. Pigment production at 20° but not at 37° is a gross and obvious example; but many properties described as characteristic are really of the same sort. The shape and structure of colonies on gelatin are, for example, largely the result of the density of the medium and the moisture of the atmosphere. Of true protoplasmic variations, which lead to characteristic differences in behavior under identical conditions, there must be again two distinct types. The first sort are due to causes operating within the cells, variations which arise between the offspring of one original bacterium when growing under approximately the same con-

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ditions. The origin of these variations, in the absence of amphimixis, must apparently be sought in inequalities of division between individual bacterial cells in the process of reproduction. Variations of this general type may be minute fluctuating variations or well-marked, sharply separated sports, or mutations. Fluctuating variations are common and mutations have been recorded by a few observers (Neumann,¹ Beyerinck,² Neisser,³ Massini⁴).

A second type of variations, distinct from these intrinsic ones, is due to the effect of special environmental conditions upon a succession of bacterial generations. These have been called impressed variations, among the bacteria. Impressed variations may be due to a direct reaction between the bacterial protoplasm and its environment as is probably the case in the exaltation of virulence by successive animal inoculations; or they may be due to a selective force acting upon fluctuating variations of the type earlier discussed. Which of these two latter sorts of variation we are dealing with in a given case it is often difficult to say.

In a recent very valuable contribution to this subject, Goodman⁵ has shown that, by a gradual process of selection of impressed variations, organisms of the *B. diphtheriae* group could be profoundly modified in regard to acid production. A culture of *B. diphtheriae* which produced an acidity of about 2.0 per cent normal in dextrose broth was inoculated into 15 tubes of dextrose broth and the final acidity determined by titration. From the tubes showing the maximum and minimum acidities, two sets of 15 tubes each were inoculated, and designated the high and the low series, respectively. Each series was carried on for 36 transfers, the tube of maximum acidity in the high series and the tube of minimum acidity in the low series being used in each case for the next transfer. At first the high and low series did not differ widely; but after 15 transfers they began to diverge. The final acidity of the high series was nearly 5.0 per cent normal while the low series finally produced an alkaline reaction.

In this important investigation it was demonstrated that a marked

¹ *Archiv f. Hyg.*, 1897, 30, p. 1.

² *Kon. Akad. v. Wetenschappen*, Amsterdam, 1900.

³ *Centralbl. f. Bakt.*, 1906, 38, Abth. 1, Ref. Beiheft, p. 98.

⁴ *Archiv f. Hyg.*, 1907, 41, p. 250.

⁵ *Jour. Infect. Dis.*, 1908, 5, p. 421.

change in a fundamental bacterial character could be brought about by a process of selection. Peckham¹ and Horrocks² similarly report that the indol reaction and other properties of the *B. coli* group may be modified by cultivation under favorable conditions. Twort³ has more recently been able to develop fermentative power in bacteria of the *B. enteritidis* type by continued cultivation in sugar media. In all these cases the modification produced is in the nature of an impressed variation, since it is the result of prolonged cultivation under special conditions. In Goodman's experiments it seems probable that the chief force at work was the progressive selection of spontaneous variations in bacterial activity. An important part may also have been played, however, by the direct influence of the environment. The organisms of his high series were exposed for transfer after transfer to high acidities and those of the low strain to low acidities. Under such circumstances there must have been a tendency to acquire or to lose a tolerance of the acid reaction as a result of the direct action of the environment.

The present investigation was planned so as to exclude the latter factor, the direct effect of the environment, and to deal with the inheritance of spontaneous variations of the fluctuating type. Meyer⁴ and Lepeschkin⁵ have recorded a tendency to the inheritance of branching in the offspring of certain cells of *B. cohaerans* and *B. Berestnewi*; and a very beautiful piece of work on this subject has recently been published by Barber.⁶ He found that elongated cells occurred rarely but with considerable regularity in cultures of *B. coli* and that by isolating these cells and breeding from them he could get a new race, constant to the elongated form and showing other characteristic differences from the parent strain. Similar, though less conclusive, results were obtained with *B. typhi*, and in one case an apparently non-spore-forming race was derived from *B. megatherium*. Barber was evidently dealing with mutations entirely comparable with those which appear in the evening primrose; and the branching cells studied by Meyer and Lepeschkin were also apparently discontinuous variations. In regard to fluctuating, continuous

¹ *Jour. Exper. Med.*, 1897, 2, p. 549.

² *Jour. Roy. Army Med. Corps*, 1903, 1, p. 362.

³ *Proc. Royal Soc.*, Series B., 1907, 79, p. 329.

⁴ *Centralbl. f. Bakt.*, 1901, 30, Abth. 1, p. 49.

⁵ *Ibid.*, 1904, 12, Abth. 2, p. 641.

⁶ *Kansas Univ. Sci. Bull.*, 1907, 4, p. 1.

variations Růžicka,¹ Conn,² Smith,³ and Sullivan⁴ have reported significant changes due to selection but the variations (liquefying power, or chromogenesis) were not measured by exact quantitative methods.

The organisms chosen by us for study were strains of the two types A and B of the paratyphoid bacillus of Schottmüller. These types are alike in fermenting dextrose with the production of gas and acid in slightly acidifying lactose media without gas formation, and in failure to form indol in peptone media. They differ mainly in their action on milk and in the vigor of their growth on media. Type A grows like the typhoid bacillus on gelatin and potato and gives a slight but permanent acid reaction in milk. Type B is more vigorous, forming a richer growth on gelatin and potato, and in milk producing first a faintly acid reaction and after four or five days an alkaline one. Type B also produces a slightly higher acidity and a larger amount of gas in dextrose broth. The property of acid formation in dextrose broth was selected for study, on account of the ease and precision with which it can be measured.

Cultures of the two types of the paratyphoid bacillus were obtained through the courtesy of Mr. B. R. Rickards, from the laboratory of the Boston Board of Health, where both had originally been isolated from cases of paratyphoid fever. Their reactions were re-examined and found to be entirely characteristic. Each culture was plated out on a series of gelatin plates, so as to get well-isolated colonies and 100 agar tubes were inoculated from 100 separate colonies of each strain. These tubes were incubated at 20° until a distinct growth appeared, and a tube of 1 per cent dextrose broth was inoculated from each. The dextrose broth tubes were called Series I (Type A), and Series IV (Type B). After 72 hours at 20° the acidity in each of the 200 tubes was determined by titration in the cold against twentieth-normal sodium hydroxid, using phenolphthalein as an indicator. Sterile tubes of the same batch of broth were titrated at the same time and their average acidity subtracted from that of the inoculated tubes.

¹ *Archiv f. Hyg.*, 1899, 34, p. 140.

² *Jour. Boston Soc. Med. Sci.*, 1900, 4, p. 170.

³ *Ibid.*, 1900, 4, p. 95.

⁴ *Jour. Med. Res.*, 1905, 14, p. 109.

The results are shown in Table 1, Columns I and IV, the figures opposite each acidity-class indicating the percentage of the 100

TABLE 1.
DISTRIBUTION OF PARATYPHOID CULTURES ACCORDING TO ACID PRODUCTION.
(Percentage of Cultures in Each Class.)

ACIDITY-CLASS Per cent Normal	TYPE A			TYPE B		
	I	II	III	IV	V	VI
1.15-1.20.....	2	1	*			
1.20-1.25.....	3	6	1		1	
1.25-1.30.....	11	8	7			
1.30-1.35.....	8	18	15			
1.35-1.40.....	13	11	14	3	1	*
1.40-1.45.....	24	26	28	2	17	1
1.45-1.50.....	22	16	18	2	11	10
1.50-1.55.....	8	6	8	7	22	8
1.55-1.60.....	7	6	8	14	13	22
1.60-1.65.....	2	2*	1	21	14	12
1.65-1.70.....				26	9	24
1.70-1.75.....				11	7	11
1.75-1.80.....				13	4	4
1.80-1.85.....				1	*	4
1.85-1.90.....					1	
1.90-1.95.....						4
Mean.....	1.41	1.40	1.42	1.64	1.55	1.63
Median.....	1.43	1.41	1.42	1.67	1.54	1.64
Standard deviation	0.49	0.50	0.43	0.50	0.59	0.57

cultures falling in that class. The same results are shown graphically in the upper section of the figure, plotted as polygons of frequency. The abscissae are acidity-classes and the ordinates indicate the percentage of cultures in each class.

The acid production for each type forms a normal curve of frequency with a mean for Type A at 1.41 and for Type B at 1.64. The median is close to the mean in each case and the Standard Deviations are respectively 0.49 and 0.50. The range for Type A is from 1.15 to 1.65; and for Type B from 1.35 to 1.85. Evidently the two types, while they overlap, vary about distinct centers.

The differences between the individual members of each set of 100 tubes may have been due in part to variations arising from some cause in the broth tubes themselves. If, however, there were any intrinsic differences in dextrose-fermenting power between the 100 single bacterial cells which formed the starting-point for each series, a part of the observed variation in acidity must have been due to these initial differences. A control experiment with 50 dextrose tubes inoculated from the same agar streak showed much less variation in acidity than was apparent in our main experiment. In this special

test (with Type B), 60 per cent of the acidities fell between 1.55 and 1.65. The wider variation in the series inoculated with strains derived from separate colonies may therefore be attributed to

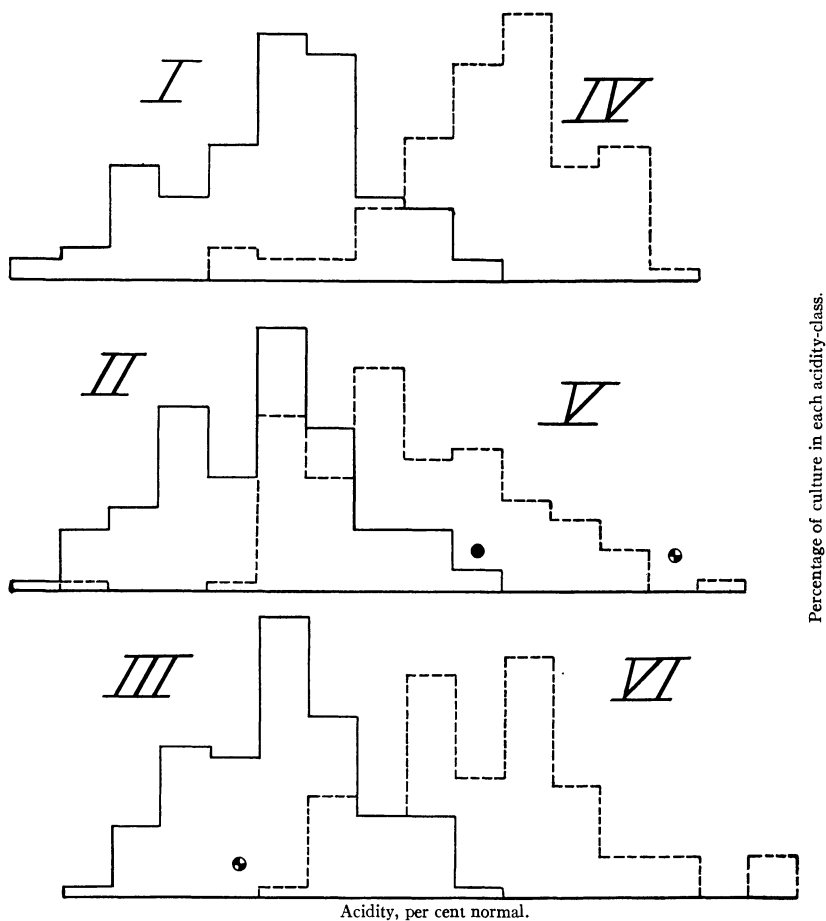


CHART I.—Distribution of Paratyphoid Cultures According to Acid Production.

— Type A --- Type B
 ● Parent strains for Type A
 ⊕ Parent strains for Type B

intrinsic differences between the parent cells of the colonies themselves. It was the inheritability of these original variations that we wished to examine. We did not therefore make further inoculations from the broth cultures themselves, in which the organisms had been

actually exposed to varying degrees of acidity. We went back to the original agar streaks and selected, first the two streaks which, when inoculated into broth, had shown the highest acidities for their respective types. These streaks were plated out and from colonies on the plates new agar streaks were made, Series II and V. The cultures in Series II were derived from the parent streak of the highest acidity in Series I, Type A (acidity between 1.60 and 1.65). Series V was derived from the parent streak of the highest acidity in Series IV, Type B (between 1.80 and 1.85). From each of these series dextrose broth tubes were inoculated and titrated as before; and the results are indicated in Columns II and V of the table and in the middle section of the figure. The whole procedure was then repeated in Series III and VI with cultures made from individual colonies derived from the parent streaks of the tubes which showed minimum acidities in Series I and IV. One of the streaks in Series I, Type A, which gave in broth an acidity between 1.15 and 1.20 was used as the starting-point for Series III; one of the streaks in Series IV, Type B, which gave acidities between 1.35 and 1.40 was the starting-point of Series VI. The source of each series, as regards acid-producing power, is indicated on the table and in the figure by a star or circle. In Series II, III, V, and VI the actual numbers of subcultures examined were respectively 88, 98, 72, and 74, instead of 100; but all figures are reduced to percentages so as to be comparable.

It is at once evident from the table and from the figure that there was no apparent modification of the character of either type as a result of selected fluctuating variations. Each curve reverted completely to the original mean of its type. Thus Series II, derived from a streak the direct inoculation of which into broth produced an acidity between 1.60 and 1.65, had its mean, not at 1.60 but at 1.40; conversely Series VI, derived from a streak which originally produced an acidity between 1.35 and 1.40, had its mean, not at 1.40 but at 1.63. The only irregularity in the whole experiment is in Series V. This series was derived from the parent streak of a tube showing high acidity; but, instead of having a high mean, it showed a mean of 1.55, below that normal for the type, with a high standard deviation. This aberration was undoubtedly due to the fact that the incubator in which this particular series was cultivated in broth was cooled below

room temperature, through an oversight in regard to the regulating apparatus. Altogether it is evident that if there were any protoplasmic differences in acid-producing power between the original cells from which the individuals in Series I and IV were derived they were only transient; and in their later descendants there was a complete reversion to the general mean of the parent strain. Taking into consideration the close relationship of Types A and B and their very slight initial differences, the constancy with which these differences were maintained is a striking example of the possible fixity of bacterial types in the absence of modifying environmental conditions.

The difference between our results and those reported by Goodman may be due to several causes. His cultures were carried through a great many transfers, so that slight initial differences were accumulated. On the other hand, the mean of 100 cultures is a fairly delicate measure and ought to detect quite minute variations of character. His cultures were exposed to the direct modifying effect of media of greater or less acidity; and this factor may be of importance. It scarcely seems likely, however, that Goodman's results could have been attained without the existence of intrinsic variations for selection to work upon. A preliminary study, in which he determined the acid-production of 103 strains of bacilli of the diphtheria group, showed indeed a variation in dextrose broth from a reaction of 0.1 per cent normal to a reaction of 4.0 per cent normal. The curve plotted from his results was a very low and irregular one with a single mean, mode and median, all between 1.75 and 1.80, and a standard deviation which we have calculated as about 0.95. If all our 538 cultures of both paratyphoid types were grouped in a single array they would fall between the limits of 1.15 and 1.95; yet in this narrow range two distinct modes would be apparent, one between 1.40 and 1.45, and the other between 1.55 and 1.60, corresponding to the two types which we have found to be apparently distinct. It seems evident from all these facts that the property of acid-production is relatively variable in the *B. diphtheriae* group and relatively stable in the paratyphoid group.